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BIOLOGICAL BULLETIN

HYDROIDS IN THE ILLINOIS RIVER.[1]

FRANK SMITH.

During the past summer (1909) while at Havana, Ill., in connection with the reopening of the Biological Station of the Illinois State Laboratory of Natural History, the writer was surprised to find at one location numerous colonies of a hydroid which presumably belongs to the genus *Cordylophora*. A superficial examination of the animal and of the literature involved has not disclosed any reason why it may not prove to be *C. lacustris* Allman which is a species of hydroid commonly found in brackish water and less frequently in fresh water.

Numerous colonies were found July 30 in a partially submerged willow thicket near the north end of Quiver Lake which is really a part of the Illinois River near Havana. The majority of the colonies were attached to the submerged portions of living willow shoots while a few were found on the leaves and stems of other plants. A later visit was made to the same locality October 16 when the water of the lake was somewhat lower and no longer covered the spot at which the July collections were made. In the part of the thicket which was still submerged, numerous colonies were found attached to dead sticks and branches that projected from the bottom toward the surface. At each visit the collections were made in water less than two feet deep and over which a considerable layer of *Lemna* had accumulated under the influence of west or northwest winds. In midsummer the colonies were in dense shade and were associated with a great variety of living organisms among which bryozoans were especially abundant.

[1] Contributions from the Zoölogical Laboratory, University of Illinois, under the direction of Henry B. Ward, No. 2.

In size, mode of branching, number of gonophores and of embryos in the gonophores the colonies are similar to those of *C. lacustris*, when found in strictly fresh water, as described by Pauly[1] and others. The main stems are but 1–1.5 cm. in height, but sparsley branched and the branches commonly bear but one gonophore which has usually not more than five or six embryos. Gonophores were present in July but not in October.

As opportunity has permitted, other places about Havana have been examined for hydroids, but thus far without success.

On August 4, during a brief visit to the Illinois River bottom-lands and lakes near Hennepin, I noticed on a partially submerged concrete wall extensive areas bearing organisms that seemed to be hydroid colonies similar to those found at Havana. As there was no time nor equipment for examination of the living material nor for its proper preservation, there is nothing at hand to serve as a basis for the identification of this form except the macerated remains of a few colonies which were scraped off and kept in a vial of water. The skeletal remains of the colonies are indistinguishable from those of the Havana species and I feel quite sure that the forms are identical and that the species is well established at two places in the Illinois River nearly a hundred miles apart.

How long this hydroid has been represented in the Illinois River is problematical, but to the writer it seems somewhat improbable that it was in the Havana region before the opening of the Chicago Drainage Canal, as for several years prior to 1900 various observers connected with the Biological Station searching persistently for all kinds of animal life found no such hydroid forms. There is at least a possibility that this hydroid may be found about the docks in the Chicago region where it may have been introduced by vessels from the Atlantic coast and then subsequently have been carried through the drainage canal into the Illinois River. More extended observations on its distribution in the Mississippi Valley are highly desirable.

UNIVERSITY OF ILLINOIS,
November, 13, 1909.

[1] *Zoölogischer Anzeiger*, Vol. XXIII., pp. 546–551.

THE ANURA OF ITHACA, N. Y.: A KEY TO THEIR EGGS.

ALBERT HAZEN WRIGHT.

For the last four years the writer has been studying the life-histories of the Anura of Ithaca, N. Y., but it will be some time before the work reaches completion. It seems best, however, to present the following brief summary of one phase of the work, in the hope that it may be of help to the numerous workers who employ Anuran eggs either for comparative or experimental embryologic purposes.

Eight species of Anura are found at Ithaca, N. Y., namely: *Rana sylvatica*, *Hyla pickeringii*, *Rana pipiens*, *Bufo lentiginosus americanus*, *Rana palustris*, *Hyla versicolor*, *Rana clamitans* and *Rana catesbeiana*.

The first five species appear from hibernation and spawn under a maximum air temperature of 43°–50° F.; the last three delay until the maximum reaches 70° F. or more. The first five usually breed from the last of March until the middle of June; the last three, from the last of May into August. All but two species, *Bufo l. americanus* and *Rana clamitans*, occupy four or five weeks for the spawning period. The exceptions may require two or three months. The number of eggs in a complement varies from 800 in *Hyla pickeringii* to 20,000 in *Rana catesbeiana*.

The eggs of three species, *Hyla versicolor*, *Rana clamitans* and *Rana catesbeiana*, float more or less at the surface of the water; the eggs of the other five are submerged. The five species with submerged eggs are first to breed. They deposit eggs with firm jelly envelopes, several eggs appearing at an emission except in *Hyla pickeringii*, where only one appears at an emission. The three with buoyant eggs breed after May 25. They deposit at the surface masses or films of eggs with loose jelly envelopes, several eggs being deposited at an emission.

At the outset the attempt to secure friutful mating with captive specimens was abandoned. The effort was rather to obtain pairs

already mated in nature. These were usually captured on night trips and were immediately taken to the laboratory, By the next morning an egg complement was ordinarily recorded. In this way a check was established upon the identification of eggs deposited in nature.

The measurements are based on fresh eggs none of which is beyond the fine morula stage. The color characters of the vitellus were taken at the time of oviposition with 7 species and not later than 6 or 8 hours after oviposition with the other species. A summary of the egg characters of each species follows in the accompanying key:

KEY TO THE EGGS OF ITHACA ANURA.

A. A single row of eggs within a long spiral string of jelly looped about plant stems, sticks or resting upon the bottom; vitellus diameter 1.0–1.4 mm.; inner envelope diameter 1.6–2.0 mm.; outer envelope diameter 3.4–4.0 mm. Egg complement, 4,000–7,000. Season at Ithaca, April 20–July..... *Bufo lentiginosus americanus.*

AA. Deposited singly or in a mass.

B. Deposited in a firm consistent mass enveloping grass stems, twigs, etc., or free; submerged; often 15–20 bunches within an area of a few square feet.

C. Small distinct inner envelope evident to the naked eye, 2.3–3.0 mm.

D. Vegetative pole yellow; animal pole brown; mass globular; vitellus 1.6–1.9 mm.; outer envelope, 3.6–5.0 mm. Egg complement, 2,000–3,000. Season, April 25–May 15..... *Rana palustris.*

DD. Vegetative pole white; animal pole black; mass plinth-like; vitellus 1.6–1.8 mm.; outer envelope 4.2–6.0 mm. Egg complement, 3,500–4,500. Season, April 10–May 15..... *Rana pipiens.*

CC. Large inner envelope apparently absent, evident only under the lens, 3.6–5.8 mm.; vitellus 1.8–2.4 mm.; outer envelope 5.2–9.4 mm.; mass globular; vegetative pole white; animal pole black. Egg complement, 2,000–3,000. Season, April 1–30..... *Rana sylvatica.*

BB. Deposited not in a hard consistent mass.

C. In small bunches or attached singly; vitellus, .9–1.2 mm.

D. Outer envelope loose, 4.0–7.8 mm.

E. Inner envelope, 1.6–2.0 mm.; vegetative pole yellowish; in small bunches (4–25) usually floating at the surface of the water, either attached to vegetation or free; outer envelope, 4.0–6.0 mm.; vitellus, 1.1–1.2 mm. Egg complement, 1,500–2,000. Season, May 20–July 1..... *Hyla versicolor.*

EE. No inner envelope; vegetative pole white; in bunches (20–100) usually attached beneath the surface of the water; outer envelope usually 5.0–7.8 mm., rarely 3.0 mm.; vitellus .9–1.2 mm. Egg complement, 500–800. Season, March 20–April 15. *Chorophilus triseriatus.*[1]

DD. Outer envelope firm, 1.4–2.0 mm.; vegetative pole never yellow;

[1] Introduced at Ithaca in April, 1909.

single or in small bunches (4–12) attached to grass beneath the surface of the water; vitellus, .9–1.1 mm. Egg complement, 800–1,000. Season, April 5–May 10.....*Hyla pickeringii.*

CC. In large loose masses; vegetative pole white; animal pole black; vitellus, 1.2–1.7 mm.; at or near the surface of the water.

D. Usually one continuous film, 1–2 eggs thick, on the surface of the water, the film's diameter being seldom 1 foot; inner envelope distinct, 2.8–4.0 mm.; egg mass usually attached or amongst vegetation. Egg complement, 3,500–4,500. Season, May 25–August 10.

Rana clamitans.

DD. Either a film 1–2½ feet in diameter or a stringy frayed widespread mass; attached to twigs or sticks; almost invariably amongst brush and at or near the surface of the water; no inner envelope. Egg complement, 12,000–20,000. Season, June 20–July 25.

Rana catesbeiana.

A NOTE ON REDUCTION IN THE MATURATION OF MALE EGGS IN APHIS.

N. M. STEVENS.

In discussing the unpaired heterochromosome in aphids, in 1908,[1] I expressed the opinion that the two heterochromosomes of the parthenogenetic generations must pair before maturation of the male-producing eggs, and separate in the maturation mitosis, one undivided heterochomosome going into the polar body while its mate remains in the egg. The only evidence which I could give in favor of this surmise was two equatorial plates where seven chromosomes appeared in the maturation of parthenogenetic eggs, instead of the eight chromosomes characteristic of the species (Plate II., Figs. 52 and 53). The largest of the seven chromosomes was evidently equal in volume to the sum of the two largest in the plate containing eight. I have never found the males of this species, the parthenogenetic generations continuing up to the time when the host plants are killed by frost. The probability is that a few scattered sexual forms occur among the parthenogenetic, and that the eggs with seven chromosomes were male-producing eggs.

In many species of aphids the same individual may produce both males and females and often parthenogenetic offspring also, making it very difficult to be certain that one has the male eggs. In the dimorphic red and green aphis found on *Œnothera biennis*, however, the rule is, that in October parthenogenetic young cease to appear; apterous mothers produce only male, and winged mothers only female offspring. Only two exceptions to this rule have been observed. Two years ago I had one brood of males produced by a winged mother, and recently, in examining sections of an apterous specimen, I found four large parthenogenetic embryos, while all of the smaller embryos were male. The change here from parthenogenetic to sexual reproduction came during a generation instead of between generations.

[1] "An Unpaired Heterochromosome in the Aphids," *Journ. Exp. Zoöl.*, Vol. VI., No. 1, Jan., 1909.

This year an unusual number of the apterous, male-producing individuals were secured. Some were fixed and sectioned, others dissected and the eggs and embryos studied in Schneider's aceto-carmine. The sections gave no favorable stages. Out of a large

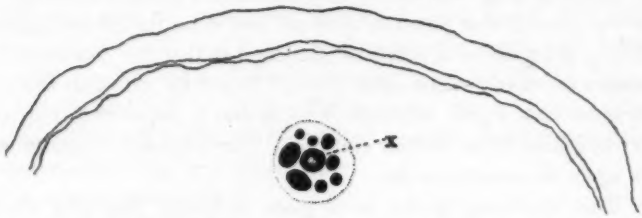

FIG. 1. Equatorial plate of maturation mitosis of male aphid egg. Outline of egg and membrane shown above. *x*, the double chromosome. Zeiss 1.5-6, cam.

number of aceto-carmine preparations, one egg was found which had the maturation spindle in metaphase. This was taken from an individual in which the older embryos were certainly male. The equatorial plate contained nine chromosomes, ten being the

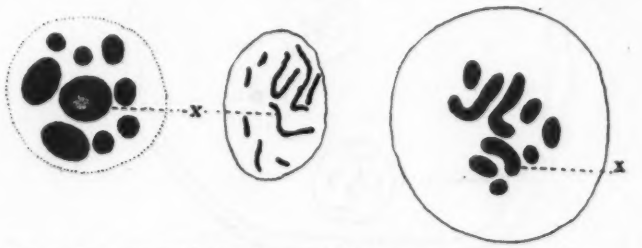

FIG. 2. Same equatorial plate drawn with Zeiss 1.5–12.

FIG. 3. Nucleus of embryonic cell (♂) in prophase. *x*, the unpaired heterochromosome. Zeiss 1.5–6.

FIG. 4. Male embryonic cell in metaphase. *x*, the unpaired heterochromosome. Zeiss 1.5–12.

number in non-sexual parthenogenetic eggs ('05, Pl. I., Figs. 7 and 12[1]). This metaphase is shown in Figs. 1 and 2. The chromosome in the center of the group is the double one. In the first spermatocyte the lagging heterochromosome is the

[1] "A Study of the Germ Cells of *Aphis rosæ* and *Aphis œnotheræ*," *Journ. Exp. Zoöl.*, Vol. II., No. 3, Aug., 1905.

second in size ('09, Pl. II., Figs. 54, 56, 57 [1]) and here it is evidently the two second in size, which have fused to form the large vacuolated chromosome *x*. No prophases or anaphases were found.

In the young male embryos many cells were in mitosis and in a few cases it was possible to count and draw the chromosomes. Fig. 3 is a nucleus in prophase, flattened so that the nine chromosomes are nearly in the same plane. It will be seen that the two longest form a pair, while the next in size is unpaired. Fig. 4 is a metaphase from another embryo. The unpaired chromosome is again the second in size.

This evidence, so far as it goes, indicates that one whole chromosome goes into the polar body of the male egg, leaving

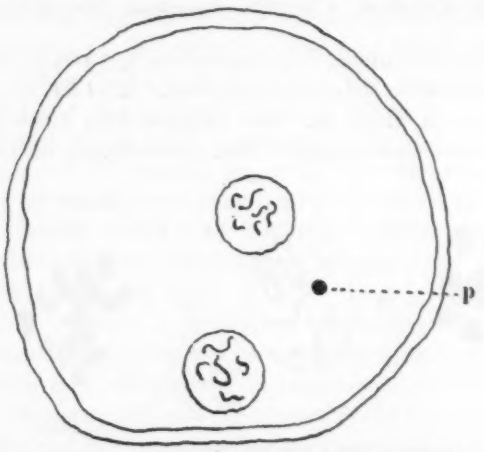

FIG. 5. Male egg showing the single polar body (*p*) and two nuclei. Zeiss 1.5–2.

the somatic number for the male individual reduced by one. Ordinarily one would not lay much stress on the conditions observed in a single egg, but considering the difficulties involved in securing favorable stages of maturation in male eggs of aphids, and the fact that the observations are in accord with Morgan's [2]

[1] "An Unpaired Heterochromosome in the Aphids," *Journ. Exp. Zoöl.*, Vol. VI., No. 1, Jan., 1909.

[2] "A Biological and Cytological Study of Sex Determination in Phylloxerans and Aphids," *Journ. Exp. Zoöl.*, Vol. VII., No. 2, Sept., 1909.

observations on male eggs of Phylloxera, it seems desirable to bring the results obtained from this autumn's collections of the *Œnothera* aphid, to the notice of those interested in the subject.

In a recent paper, entitled "Mendelian Inheritance of Sex," Hagedoorn has quoted me as authority for a statement that male aphid eggs give off two polar bodies. I have found only one polar body in male and other parthenogenetic eggs, and have so stated the fact with all the evidence at hand ('05, Pl. II., Figs. 17 and 18, and text p. 317). Fig. 5 shows one of many early segmentation stages of male eggs found in aceto-carmine preparations, showing only one polar body

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November 25, 1909

A METHOD TO TEST THE HYPOTHESIS OF SELECTIVE FERTILIZATION.

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The hypothesis of selective fertilization has played an important rôle in several recent theories of sex determination and to some extent also in modern Mendelian speculations. It has been assumed for example that there are two classes of spermatozoa and two classes of eggs — male and female eggs and male and female sperm — and that fertilization is reciprocal in the sense that a male sperm can only fertilize a female egg and that a female sperm can only fertilize a male egg. On the other hand the more commonly accepted view is that any sperm can fertilize any egg.

Until this question is settled by direct observation or by experiment these two alternatives will continue to make uncertain our interpretations.

To put together one sperm and one egg would seem to be the simplest way to test the question. The operation may not in itself present insuperable difficulties but the chance of the spermatozoön reaching the surface of the egg is so small as to make the attempt rather hopeless; for there is no evidence to show that the spermatozoön is attracted towards the egg. The work of Buller, in particular, shows that accident alone determines the contact between the spermatozoa and the membranes, or the jelly, of the egg.

By means of the following simple method we have found it possible to study the problem of selective fertilization. We feel that while the number of cases here recorded is too small to settle so important a question, its application on a larger scale and on other animals should furnish conclusive evidence for or against selective fertilization.

We wish therefore at present to lay more emphasis on the possibilities of the method than on the certainty of demonstration from the number of recorded cases, and hope that others

may be led to study this important question by the same method.

We made use of the eggs and sperm of the mollusk *Cumingia*. The spermatozoa are quite large and can be readily seen with moderate magnification. The eggs are small so that the entire exposed surface can be watched closely. The eggs just laid were put into a drop of water on a slide, and a cover slip added. The cover slip was sufficiently supported so that the eggs were not too much compressed. It was advantageous to apply pressure because otherwise the spermatozoa may reach the egg above or below the horizon of observation; such sperm are lost to sight as a rule and their fate remains uncertain. Even with moderate pressure the spermatozoön sometimes slips in between the egg and the cover slip (above or below the horizon) and are lost to sight. In such cases further observation is worthless.

By means of a fine pointed pipette a small drop of water containing not too many spermatozoa was introduced at the edge of or under the cover slip. From the point of insertion the spermatozoa swim out in all directions and at some distance from the starting point the path of a single spermatozoön could be easily followed. The only way in which we were absolutely certain of seeing the first spermatozoön that reached the egg was to focus on an egg and wait until one came in contact with the egg.

It was seen that many spermatozoa swim past the egg and show no evidence of turning towards it, but those whose previous path was such that they ran into the jelly around the egg, bored into the jelly and often reached the surface of the egg. Whether after a spermatozoön has entered the jelly it ever turns towards the egg (if it did not have this orientation at the time of contact) is difficult to determine with certainty, but it is certain that spermatozoa may bore through without turning owards the egg. Some of the attached spermatozoa may show alternate periods of rest and activity, and in consequence change their position several times, and even end by entering the egg, but there is no evidence that one position is more directed than another.

The successful spermatozoa are those that strike the egg "head-on," and bore directly towards the surface. When the surface is reached the end of the spermatozoön appears to enter

the outermost layer of the egg. As a rule a pause follows, and it may take the spermatozoön from five to twenty minutes to disappear within the egg. The penetration is, as a rule, at first slow but later the spermatozoön may enter quite suddenly. Whether the sperm bores its way into the egg, or whether there is first a reaction between the surface of the egg and the spermatozoön so that the egg also takes a part in the process need not be discussed here, but it is important to note that no spermatozoa enter except those that stand with their long axis vertical to the surface and pierce the surface with the tip of the sperm head.

Our observations show that the first sperm that fulfills these conditions is received. In some of these cases a second sperm came in contact with the egg after the first had come in contact with it. In all such cases the first sperm only penetrated. There was no evidence in favor of selective fertilization, since in all forty cases the first sperm that approached in the normal position was the first to enter. It is highly improbable that forty times, this first sperm was the one suited to enter (assuming that two kinds exist) when there is no evidence that the eggs attract the sperm. Our general conclusion from the data here presented is that in this case selective fertilization does not occur, and since *Cumingia* is unisexual the temptation is to generalize this statement to include all such forms. Whether this extension is warrantable or not the fact remains that in this, the only case so far tested, the evidence is opposed to the hypothesis of selective fertilization.

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November 20, 1909.

THE ANATOMY OF THE STYLETS OF CAMBARUS AND OF ASTACUS.

E. A. ANDREWS.

In all the crayfishes, *Cambarus* and *Astacus*, of the northern hemisphere, the limbs of the first and second segments of the abdomen of the male are modified in a peculiar way and are used as instruments to transfer the sperm from the defferent ducts to the outside of the body of the female. These limbs we will call the first and second pairs of stylets.

In *Cambarus* it was found (1, 2) that the stylets place the sperm within a special sperm receptacle in the shell of the female. In *Astacus*, as far as is known, the stylets deposit the sperm over the shell of the female in secreted tubules, or spermatophores.

While the stylets are much alike in the two genera the following study shows that the parts directly concerned in the passage of sperm present two stages of perfection, those of *Cambarus* being the more specialized. The first pair of stylets are firm, awl-like structures, which in *Astacus* are evidently comparable to a rolled in plate, while in *Cambarus* they seem solid with only a superficial groove. But we now find that this groove is the outlet of a concealed tubule and that in *Cambarus* also the organ may be regarded as a modified plate.

As the second stylets are much alike in the two genera and have a subordinate rôle to play in the process of sperm transfer we will consider chiefly the first pair.

It is a remarkable fact that the first stylets in these crayfishes present specific differences so that the systematist relies upon the forms of the stylets as an important aid in the description and identification of species. The figures of Hagen (3) and of Faxon (4) show the great amount of diversity in proportion and in character of termination in many species of *Cambarus*. But despite this diversity in form the following description of the first stylets in four species taken at random and representing four of the six subgenera of *Cambarus* makes it probable that in all species the stylets have the same essential anatomy and use.

We will first describe the stylets of *Cambarus* and then those of *Astacus*.

In these reduced limbs (Figs. 1 and 2) we may distinguish the base (*B*), the neck (*N*) and the spiral (*S*) which is the region with a somewhat spiral lengthwise groove bounded by hard rounded edges that run out to form the two tips of the whole organ. It is this bifid appearance of the limb which has been most emphasized in descriptive work. One of the tips may in this species be called the spatula (*Sp*) from its shape. The other tip (*C*) may be called the canula, as it is a termination of a tube and is inserted into the sperm pocket of the female and allows the sperm to pass out of its tip. Of the two apparent tips of the limb, one, the spatula, is thus a side outgrowth of minor importance; the other is the real morphological and physiological end of the organ and of fundamental value.

The groove that runs along the length of the spiral region begins at an orifice (*Or*) and ends at the extreme tip of the canula.

We may regard this groove as dividing the spiral region into two parallel portions, the external mass (*Ex.m.*) and the median mass (*M.m.*), external and median being used with reference to the median plane of the entire animal.

In *Cambarus virilis* (Figs. 1 and 2) the stylet is exceptional in the great elongation of the spiral region, the spatula being very much prolonged and the canula a curved, ovipositor-like structure. The tufts of setæ at the junction of neck and base and upon the median mass near the orifice are also long.

Cross-sections (Fig. 3), at the level 3 of Fig. 2, show that the external groove of the canula passes deep into the interior and has its inner end partly cut off as a tubule by a ridge or shelf which projects like a valve from the side of the groove. Serial sections show the same general facts throughout the length of the spiral. There is thus a continuous tubule from the orifice to the tip of the canula.

While the stylet has most of its exoskeleton firmly calcified, as represented by the black in the section, the tips of the spatula and canula are partly horn-like. In the median mass this horn extends some distance toward the base, as represented in the dotted area in the figure. The shelf that overhangs the tubule

is also of horny and not of calcified material and thus may the better make a closure of the tubule.

The living tissue of the stylet was found to be a loose areolar mass full of blood spaces and covered by a thin epidermis that makes the exoskeleton. This tissue filled the vacant space in the external mass in Fig. 3.

Excepting the muscles in the base that move the whole limb upon the body there are no muscles within the stylet, but on the other hand there are largely developed glands in the swollen proximal parts of the spiral. These glands discharge through the shell into the tubule not far from the orifice.

In *Cambarus diogenes*, which belongs to the subgenus ~~*Bartonius*~~, Cambarus the stylets (Figs. 4 and 6) have a very different appearance owing to great shortness of the terminal portion of the spiral. The base and the neck remain much as in *C. virilis*, but the spatula and the canula are very short and thick with the tips turned up-dorsally (Fig. 4). They are also much flattened, and are thus very narrow as seen from the posterior face (Fig. 6).

Practically the whole length of the canula and much of the spatula are horny. A section across the canula (Fig. 5) shows the shelf from the external mass (*Ex.m.*) and the isolated bottom of the groove. It also shows that the median mass (*M.m.*) has exaggerated the tendency seen in *C. virilis* (Fig. 3) to grow over the groove, to such an extent that it runs over the shelf of the external mass and so makes the closure of the tubule a very complete one.

The horny tip of the canula shows its finer structure more readily than in *C. affinis* and we see under higher magnifications that the horny substance presents lengthwise striations on the surface, which at the tip give place to areolations suggesting scales. Possibly this slight roughening of the tip of the canula may be of some use in cleaning out the orifice of the sperm receptacle.

In the southern form, *Cambarus Clarkii*, the first stylet is the antithesis of that of *C. virilis* for the terminal parts of the spiral (Fig. 7) are so greatly shortened as to form a flat mass that is largely horny and though bent upward, somewhat as in *C. diogenes*, more complicated at its tip.

The setæ are short but form very long rows. Those of the base extend along the neck far up onto the external mass and those of the median face run nearly its whole length. There are also some additional setæ upon the dorsal face near the tip as seen in Fig. 8.

In following the groove (Fig. 7), we find that after it passes the very slender and insignificant spatula it turns ventrally to end on a blunt protuberance indicated in the dotted area in Fig. 7. There is left a large protruding mass external to the spatula, which is indicated in parallel shading, and which is evidently part of the median mass that does not extend to the very tip of the groove. Thus the tip of the stylet is bifid; the blunt part to the right in the figure is the canula and the part to the left is a sharp blade formed from the median mass beyond the spatula and this we will call the scalpel, from its shape and its probable use in opening the very firmly closed and constricted slit of the annulus in the females of this species. The scalpel is apparently well placed to cut open the orifice of the annulus (see *1*, Fig. 24).

The character of this condensed terminal region is better seen in the enlarged view of the external face of the tip (Fig. 8). The scalpel, to the right, bears the above-mentioned setæ at its base and has a sharp convex edge.

It is set off from the canula by a depression. The canula itself, as seen behind the outer parts of the setæ, is slightly bifid in this preparation but in dried specimens it ends with a terminal orifice. While the stylet has two elastic tips the canula (*C*) and the scalpel (*Sc*) they are so near together that both might readily enter the orifice of the annulus.

On cutting sections of this unusual stylet we find that the groove has its bottom cut off by a shelf (Fig. 9), just as in the other species. But the tubule so formed is so minute and so deeply buried that it is easily overlooked. A section (Fig. 9) taken near the base of the spatula at *9* (Fig. 7) shows the median mass so extended as to make a sharp blade, the scalpel (Fig. 8). And moreover the shelf that arises from the external mass, at this level, points away from the median mass, so that the section is not readily compared with that of *C. virilis* (Fig. 13). In sections the horny shelf can be traced down from the free edge of

the end of the canula and represents the edge of the external mass along the side of the groove, but before the orifice at the base of the spiral is reached the shelf disappears and does not continue on as part of the lip of the orifice.

Within the minute tubule a small number of sperms were seen.

The use of these organs in the processes of sperm transfer was seen to be the same as in *C. affinis*. In brief the phenomena were: The hooks of the third legs hold the male firmly to the female. The male holds the claws of the female. The first and second stylets are locked together and the fifth leg is crossed. The pleopods are swung back and forth. The second stylet glides a millimeter or so up and down the first, with its wedge in the groove. Occasionally jerks of the base of the abdomen make slight hammering thrusts of the tip of the stylet-complex. While both stylets present their tips to the slit of the annulus it seems difficult for both to enter at once since the slit is median and not transverse. The exopodite of the second stylet shows some slight twitching movements. The female pleopods of the first somite extend over the annulus and touch the setose palp of the stylet. On removing such a conjugating male and placing the fifth leg across to support the locked stylets, sperm issued from the hole at the tip of the canula and in a few minutes sperm came out of the tips of both papillæ. In separating a pair one stylet was very firmly fastened in the annulus and tended to pull the annulus away with it. This stylet had shoved the annulus up into the body of the female as far as possible. This attached stylet was on the same side as the crossed fifth leg.

Cambarus Montezumæ is a representative of the subgenus *Cambarellus* from Mexico, and should present, in some respects at least, a more nearly ancestral state than the above species.

Nothing is known of the conjugation habits, as the species is known only from preserved specimens. The male has two hooks on each side, and presumably both are used as is the one in *C. diogenes*. These hooks (Fig. 10) are on the second and the third legs and are like those of *C. affinis* but less blunt. Those of the third legs are the longer, sharper and more specialized in form.

The stylet (Fig. 11) is short and simple, with the usual tuft of setæ absent from the median face which is very wide and flat.

The spatula (*Sp*) is very large and hollowed on the median face to form a wide spoon. The canula (*C*) is sharp-pointed and somewhat curved, with a horny tip that plainly shows the groove running to it and opening by a hole. There is also a new outgrowth (*L*) that has the form of the spatula of *C. virilis* but arises from the external mass, half way between the origin of the spatula and the tip of the canula. This new outgrowth we will call the ligula.

Cross-sections of this stylet (Fig. 12 at the level 12 of Fig. 11) show the presence of a shelf that cuts off the bottom of the groove as a tubule, similar to that in *C. virilis*. This shelf has the same yellow, homogeneous appearance. This section shows the long flat extension of the median mass that forms the base of the spatula and the shelf at the bottom of the overhung groove.

In the section (Fig. 13 at the level 13 of Fig. 11) we see the orifice and in the median mass (*M.m.*) glands with one of the tubules discharging through the exoskeleton into the groove.

This specimen seems to have been about to shed, so that the exoskeleton is represented rather schematically in the sections, as it was broken or laminated.

Turning now to the genus *Astacus*, to which all the European crayfish belong, the process of sperm transfer is known only from the brief accounts of Soubeiran (5), Chantran (6) and Huxley (7). From them it appears that the males seize and turn the females and mount them, but the subsequent stages differ from those in *Cambarus* in the fact that there are neither hooks nor annulus, and thus no transfer of sperm to any sperm pocket; on the contrary, the sperm passes out of the stylets onto the sternal surface of the female in the form of spermatophores. These subsequently liberate the sperm at the time of laying in some unknown manner that Whitman (8) states was referred by Leuckart to the compession of the walls of the spermatophores and by Meyer to the action of the secretion that fastens the eggs. That the same general method is followed by the *Astacus* of the west coast of the United States seems undoubted from the similarity in the anatomy of the organs concerned and from the following observations.

Amongst female crayfish of the species *A. leniusculus*, received

from Oregon in February, or in October, 1904, there were a few that presented the remnants of spermatophores on the ventral surface of the thorax. In the best marked case these were some hundred empty tubes, 3 to 4 mm. long, and more than .25 mm. thick, of red-brown color, stuck close to the shell of the female for the most part, though some had one free end standing up about a millimeter into the water. Most were laid down carefully side by side in groups. Some few were twice the usual length. A few were on the base of the second leg, on one side; more were at the base of the third leg and close to the opening of the oviduct. Still more were at the bases of the fourth legs and between them just anterior to the annular plate, onto which two spermatophores extended. Two were on the sternum between the fifth legs. The entire collection, in a sketch, forced one's attention to the fact that they had either been originally placed in depressions and angles where they would not be readily rubbed off, or else that these seen were the survivors that had escaped removal after more unprotected spermatophores had gone. Each spermatophore had its tips greatly contracted, as if a soft material had shrunk more at the end, somewhat like egg-cocoons of earthworms.

Thus the male of the American crayfish *Astacus* must deposit the sperm in tubes over the ventral side of the thorax of the female, and not introduce it into any special cavity.

In comparing the sperm-transfer apparatus here with that of *Cambarus* we find greater simplicity, as was to be expected for the performance of this less specialized mode of transfer.

The first stylet of this *A. leniusculus* (Fig. 14) is like that of the English *Astacus*, as figured by Huxley (7) in the main, while also being like the stylet of *Cambarus*. The base is simple and without the specialization of form to nicely accommodate the second stylet, but the ridge along the neck bears setæ. The spiral is obviously a hollow cone or tapering scroll with a very wide orifice between the long external, and the shorter median mass. The groove is much more open than in *Cambarus* and the whole organ is less rigid and seems as if well made for a mere conduit and not for an organ to be forced into a hard slit. The stylet is not noticeably bifid and the rather blunt tip is the canula

while the spatula and all other lateral outgrowths are absent. Huxley says of *A. fluviatilis*, "terminal half of the appendage is really a broad plate, slightly bifid at the summit, but the sides of the plate are rolled in in such a manner that the anterior half bends around and partly encloses the posterior half. They thus give rise to a canal, which is open at each end, and only partly closed behind." In *A. leniusculus* this overlapping of the one part by the other has proceeded much farther, so that in Fig. 14 we see the anterior, or median, mass has covered in and concealed the external mass through all the terminal extent.

In sections this extensive inwrapping becomes at once patent. The section 15 shows a widely open canal closed in not only by the rolled median mass but internally by an opposite rolling of the external mass; that is, the hypothetical plate, of which the terminal part of the stylet is composed, has both its edges rolled in, first, the external edge and then the median edge outside the other. Both flaps come so close together that near the tip of the canula 15 (Fig. 14) the central tube is well shut off from the water (Fig. 15). Contrasting this with *Cambarus* (Fig. 3) we see that in both cases there is a horny plate inrolled, but in *Astacus*, this is much like the rolling up of a sheet of paper, while in *Cambarus* it is the buckling up of a thickened mass whose edges meet over a groove. *Cambarus* shows the derived, the more special, the less mechanically direct sort of inrolling.

Farther down the stylet (at 16, 17, 18, of Fig. 14) the rolling is more and more imperfect (Figs. 16, 17, 18). These figures show the rather thick calcified shell and the usual connective tissue, but the absence of glands is conspicuous. Also the edge of the external mass becomes specialized as a sharp shelf that overarches the large central canal, while the enveloping median mass still overlaps the external mass and makes the closure of the canal a more firm one.

In Fig. 17 there is a complexity of the canal that exists for a short distance and may prove to be of some significance when the process of sperm transfer can be studied. There is a narrow side-slit from the groove, to the right in the figure, which is made by special thickening of the shell of the external mass. That is, there is a ridge along the bottom of the groove. With this ex-

ception, there is nothing to suggest the minute inner part of the groove of *Cambarus* and the large hole covered by the shelf must be the homologue of the tubule of *Cambarus*.

The obvious suggestion that the canula is derived from a rolled plate is unfortunately supported by no actual observation, though the few following facts regarding the development of the stylet in *A. leniusculus* show a simple beginning that may well later suffer a process of inrolling.

A larva 19.5 mm. long, shedding from the fourth to the fifth stage, showed on the cast the two minute papillæ seen in Fig. 19, growing toward one another on the ventral ridge of the sternum of the first abdominal somite. That these are the first stylets of the male is indicated by the fact that in larvæ killed in the middle of July and presumably in the fourth and fifth stages nine showed no outgrowths and were probably females while six showed outgrowths similar to these in Fig. 19. These little stylets differed much in the different males.

In larvæ from 20 to 26 mm. long the stylets differed in size and form from the state shown in Fig. 19 to that shown in Fig. 23. The state of advance of the stylet was not parallel to the length of the larvæ, thus a male 26 mm. long had the stylet much as in Fig. 19, while one 25 mm. long had them as Fig. 23. A male 20 mm. long had the stylets shown in Fig. 20 ; they were somewhat flattened papillæ pointing toward one another. Fig. 21 shows the left stylet from a male 23 mm. long and Fig. 22 that of a similar male. The most developed stylet (Fig. 23) is not only flattened but its posterior face is somewhat concave and shows on its median edge, to the left in the figure, a slight notch to represent the future neck (compare Fig. 14), while the median edge is thickened as if it might grow up to form the enveloping median mass to cover over the flattened or concave part that would be the groove. The whole organ is then a stiff flat spoon and is remarkably like the stylet of the American lobster in miniature.

At the tip of the stylet is a minute protuberance tipped with a spine and suggesting a sensory function (Fig. 23). This was found on the stylet of the opposite side, but not in any of the stylets of other males, which were all less advanced.[1]

[1] The general proportions of the longest 26 mm. male may be seen from the following measurements: The length of antennæ 26 mm., the chelæ 18 mm., the width

While the first stylets of both *Cambarus* and *Astacus* show neither in their anatomy nor in their ontogeny any sign of derivation from the typical forked crustacean limb this is not the case with the second stylets. These remain forked in the adult and we shall see that they arise from the modification of an ordinary pleopod by the addition of a lateral outgrowth, while the first stylet would seem to be derived by the dropping out of part of the typical limb and by the condensation of the rest.

The second stylet in *Cambarus* bears a setose exopodite and endopodite that are fringed with setæ and contain muscles that move these forks of the limb upon the basal part. In the development of the larva there is added to the simple limb an outgrowth from the endopodite that finally becomes the peculiar excrescence characteristic of the second stylets of the crayfish. These outgrowths are applied by the adult male against the orifice of the first stylet and play an important accessory rôle in the processes of sperm transfer.

In *Astacus* the second stylet (Fig. 24) is much like that in *Cambarus*. It has a slender exopodite (*Ex*) and a wide endopodite (*En*) that ends in a setose filament (*Fl*). But the lateral outgrowth, or excrescence, is different. In *Cambarus* this part may be called the triangle and it ends in a rounded free edge that is inserted into the groove of the spiral of the first stylet during sperm transfer. This thick edge is somewhat comparable to a radius bone and ends with a hollowed head. Distal to this radius the triangle is continued as a pyramid, bearing setæ: this pyramid we call the "wedge."

But in *Astacus* (Fig. 24), the triangle (*Tr*) suggests an extinguisher in form since the wedge (*W*) is a direct continuation of the edge of the head of the radius in the form of a curved plate as indicated in the smaller figure to the right. The wedge (*W*) is not a pyramid at all but a thin, rather pointed plate curved around a deep depression to join the edge of the radius (*R*) as shown in Fig. 24.

of thorax 8 mm. (in alcohol), of telson fan 11 mm., of telson 3.5 mm., of setæ on telson 1.5 mm., number of joints in antennæ 75 plus. The stylets were about 2 mm. and the second stylets 4 mm. apart. There was a rounded area and a dim white organ within base of fifth leg, indicating the defferent ducts of the male, probably.

From the state found in this *Astacus*, the more "extinguisher-" like shape found in the English *Astacus* could be formed by a process of simplification, or reversal of specialization, just as is true for the first stylets. The above mentioned larvæ of *Astacus leniusculus* furnished but meager facts bearing upon the ontogeny of the second stylet, but this is enough to establish the existence of an early modification of the median edge of the endopodite to subsequently form the triangle or scroll. The second pleopod is at first like the following ones and only gradually takes on the specializations that make it an accessory sperm-transfer organ. The earliest detected modification of the endopodite was a slight groove followed in larger larvæ by an elevation on which the same groove was seen. How this groove on an elevation gives rise to the triangle remains for study of later stages to decide.

In Fig. 27, which is the anterior face of part of the second pleopod of a male, there is a marked protuberance on the median side of the endopodite, and this contains a lateral groove. In some other males the groove was present, but not the elevation. Thus Figs. 25, 26 represent the anterior and the posterior faces of the edge of the endopodite of a male 23 mm. long, showing only the exoskeleton and the plumose setæ. The groove is a transverse pit which ends abruptly on the anterior face of the endopodite; it is bounded distally by a slight lip-like transverse ridge standing out into the water.

There is thus a transverse pit on the median face of the endopodite at the region that will later be part of the triangle (compare Figs. 27, 24). The cells of the epidermis were small and ran in as a single layer to line the pit and extend into the lip as a solid mass.

In the more advanced stage Figs. 27, 28 this same pit is on a decided elevation. The pit is a transverse slit still lined with epidermal cells (Fig. 28), but its distal edge is no longer a lip but only part of the general elevation. This male was the one having the advanced first stylets seen in Fig. 23. The posterior view (Fig. 28) is intended to show the epidermal cells in surface view as well as in optical section and also the fact that the pit opens gradually onto the general level on this posterior face, while on the anterior face it ends abruptly at a steep wall lengthwise of the endopodite.

No doubt the general elevation later becomes the triangle and is comparable to the knob found in *Cambarus*, but the meaning of the lateral pit is problematical. Possibly it is the forerunner of the cavity at the end of the triangle which gives it the extinguisher-like form, and which being still more prominent in the English *Astacus* may be an old trait that would more likely find expression in the older genus, *Astacus*, than in the newer one *Cambarus*.

By way of summary we will state that the first stylet of these crayfishes, *Cambarus* and *Astacus*, has the anatomy of a pleopod that has lost both its biramous form and its intrinsic muscles and has become a nearly closed tube. In its physiology it is essentially a tube to transmit the sperm from the male to the female.

The ontogeny throws little light upon the phylogeny of the organ since at its first appearance in the larva it is already a simple papilla, which in *Astacus* becomes a flat plate that then rolls in to form a tube while in *Cambarus* it forms a tube by thickening of the edges.

The apparent simplicity of the first stylet in *Cambarus* misled Hagen (3, p. 17) to regard it as having lost its channel save for the external groove, while in reality there is a functional inner tubule.

The anatomy of the first stylet of *Cambarus* gives a firm basis for the interpretation of the various terminations of this organ as exhibited in different species and made use of for detecting genetic relationship as well as specific characters. It will be necessary to restudy the stylets of all Cambari to determine in how far the accepted morphological division into "inner" and "outer" parts is a sound basis for comparisons. In each species the canula, or real termination of the organ, must be distinguished, and the various secondary outgrowths classified as to their origin from the two sides of the groove that ends at the tip of the canula. With this knowledge a more scientific understanding of the genus may be possible. The "outer" part seems to be the canula or real end of the organ and the "inner" part only a lateral outgrowth from one side of the canula. The two are not of equal import.[1]

[1] In practice stains that enter the canula tubule will aid in recognition of the canula without the need of sections.

The second stylets of *Astacus* and of *Cambarus* have the value of pleopods that have merely added a lateral outgrowth which arises in larval life and serves as an accessory organ in sperm transfer.

It is evident that the stylets of the species of *Cambarus* studied are much more highly specialized than the stylet of the American *Astacus* studied and this in turn seems less generalized than the *Astacus* of Europe.

The stylets of *Cambarus* could be readily derived from those of *Astacus* by specialization. The addition of glands, the strengthening of the shell, the refinement of the conducting tubule and the perfection of the accessory stylet in *Cambarus* may all be regarded as correlated with the presence of the annulus and sperm pocket in the females in this genus: the more accurate apparatus of the male *Cambarus* being used for a much more specialized task.

The first stylets in both genera might be derived from a flat stylet similar to that in the lobster where, probably, the two, right and left, are used at the same time to fill the sperm receptacle.

Upon this assumption we would regard *Astacus* as having lost some sort of a sperm receptacle which has been retained and perfected by *Cambarus*.

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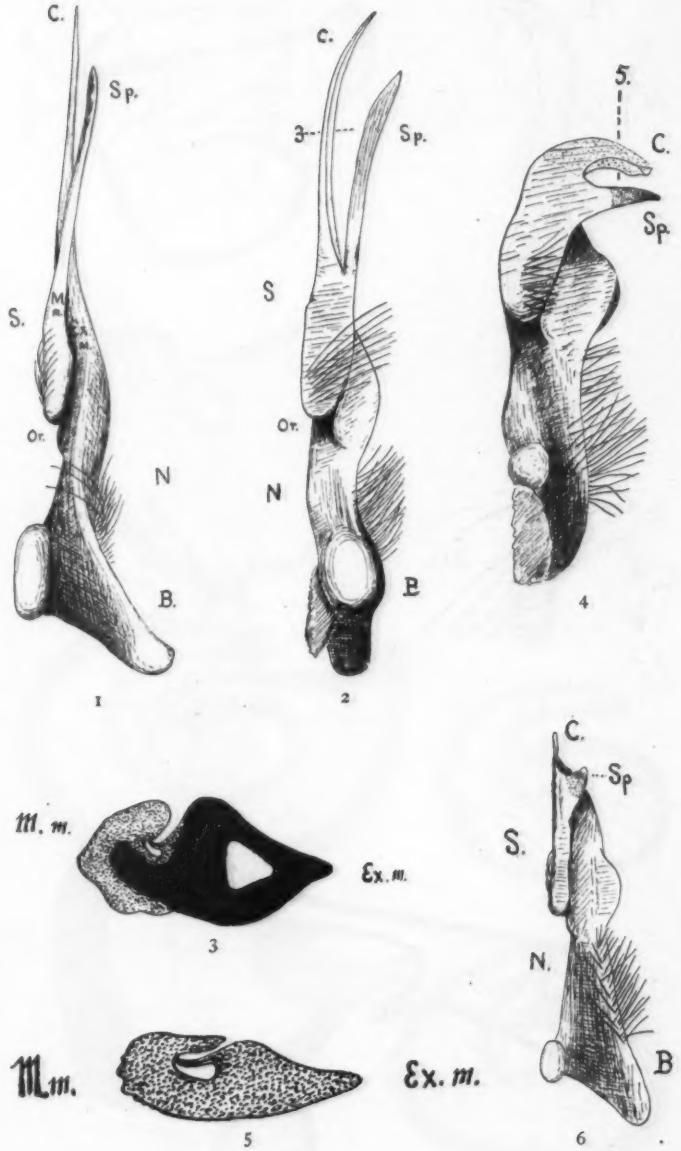
Explanation of Figures.

Fig. 1. *Cambarus virilis:* 1, posterior face of left stylet of adult, $2a_0$; 2, median face of the same, $2a_0$; 3, cross-section of the stylet at the level, $3ff$, Fig. 2, $2A$. *Cambarus Diogenes:* 4, median face of left stylet, $2a_0$; 5, section across the above at the level, 5, $2A$; 6, posterior face of the left stylet, $2a_0$. *Cambarus Clarkii:* 7, median face of left stylet, $2a_0$; 8, enlarged view of external face of tip of left stylet, 2, 90 mm., A; 9, section across stylet at level, 9, of Fig. 7, $2A$. *Cambarus montezumæ:* 10, bases of second and third left legs, showing hooks, of male 30 mm. long, anterior view, $2a_0$; 11, median face of left stylet, 2, 90 mm., A; 12, cross-section on the level 12 of Fig. 11, $4A$; 13, cross-section of same on level 13, $4A$.

Fig. 2. *Astacus leniusculus:* 14, posterior face of left stylet turned to show somewhat of the median face, $2a_0$; 15, section across the above at the level 15 of Fig. 14, $2D$; 16, cross-section at the level 16 of Fig. 14, 2, 90 mm., A; 17, cross-section at the level 17 of Fig. 14, 2, 90 mm., A; 18, cross-section at the level 18 of Fig. 14, 2, 90 mm., A; 19, papillæ, or stylet on the first abdominal somite of larval shell passing from the fourth to the fifth stage. Length of body, 19.5 mm., 2, aa; 20, stylets of a male 20 mm. long, 2, 90 mm., A; 21, left stylet of a male 23 mm. long, $2A$; 22, stylet of a male like the last, $2A$; 23, left stylet of a male 25 mm. long, posterior face, $2A$; 24, second, or accessory, stylet; anterior face, somewhat turned to show the external face in part, $2a_0$; small figure to the right is the enlarged end of the radius; 25, part of the edge of the endopodite of the accessory stylet of a male 23 mm. long, anterior face, $2D$; 26, posterior face of the same, $2D$; 27, anterior face of the endopodite of the accessory stylet of a male 25 mm. long, $2A$; 28, cell outlines over elevation and pit of the edge of the endopodite of above stylet, $2D$.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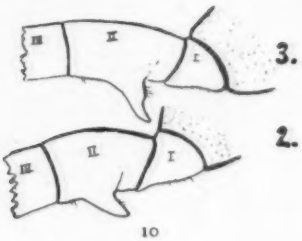

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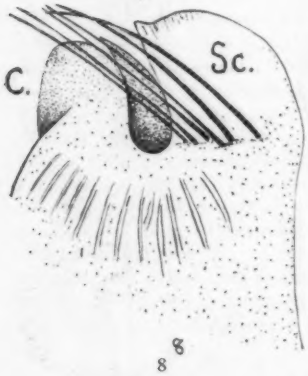

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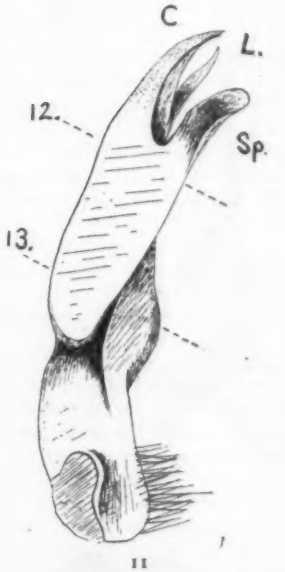

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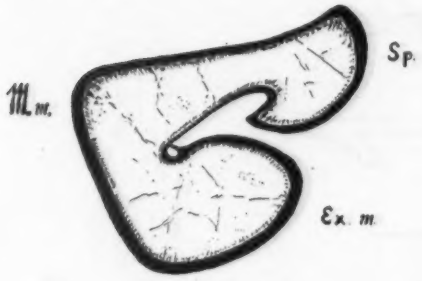

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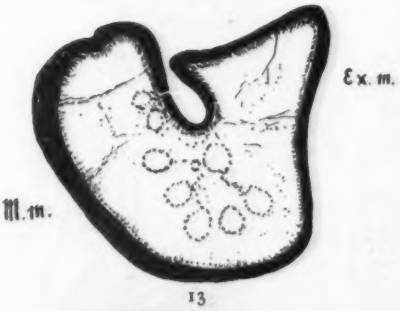

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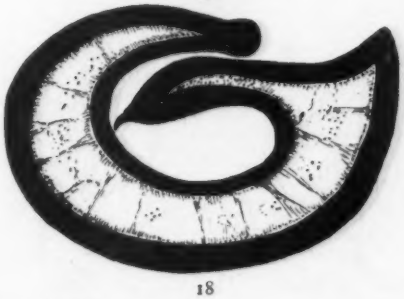

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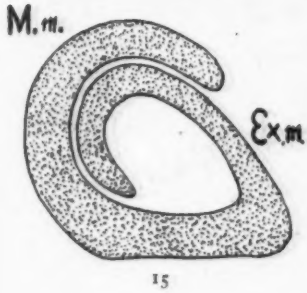

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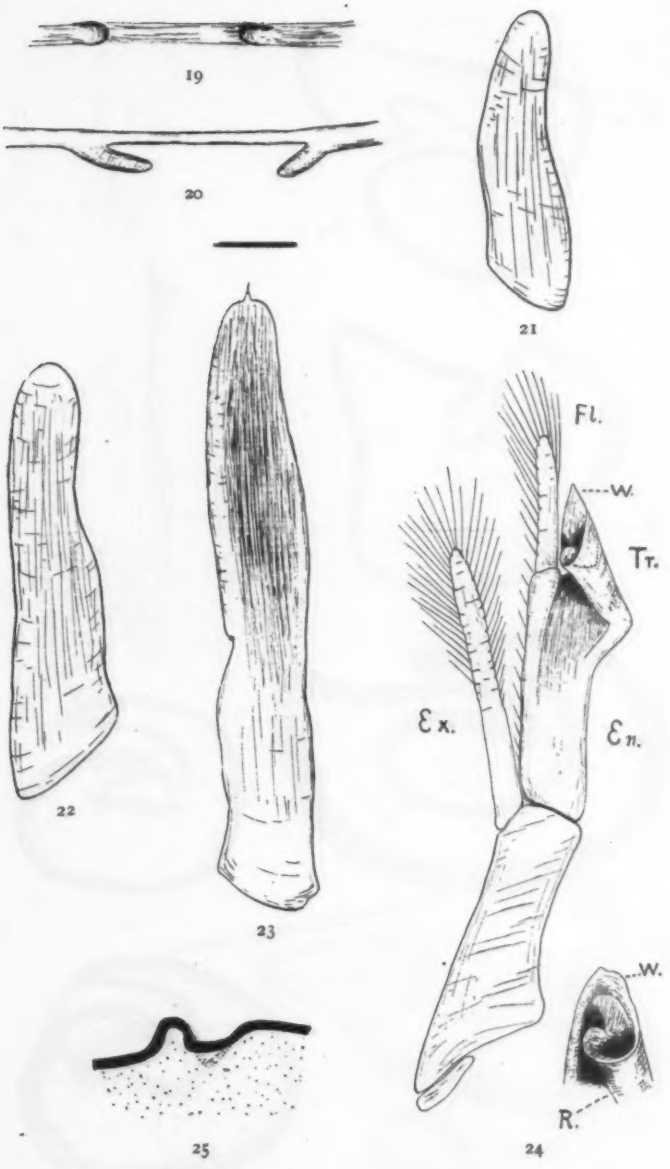
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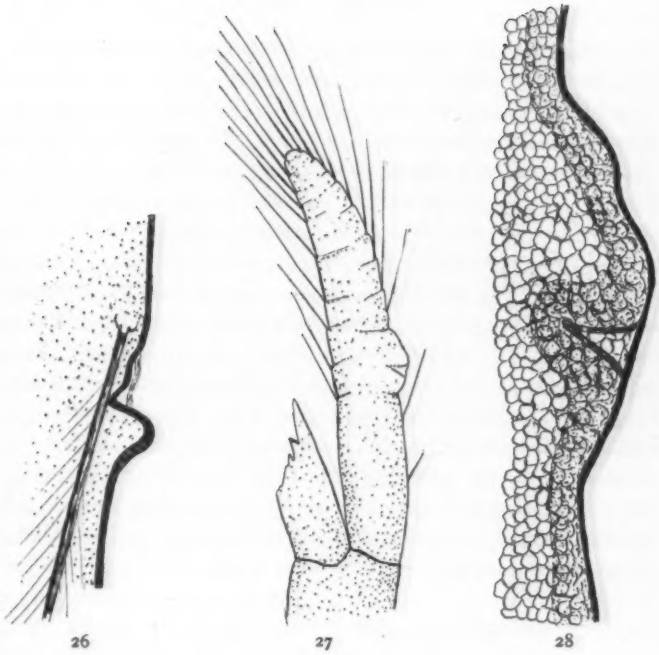

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